

Structure and cleavage of monosodium urate monohydrate crystals

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The structural study of monosodium urate monohydrate, as the principal component in gout stones, reveals that a simple and biocompatible way to breakdown the crystals into polymerised molecules at pH of 7.4 (the acidity of normal human blood) is to peel off them along the [001] direction by sonication.

Gout is commonly defined as “a crystal deposition arthropathy caused by deposition of excess uric acid crystals in joints and soft tissues”.¹ Uric acid is a substituted purine with the formula $C_5H_4N_4O_3$, also known as 7,9-dihydro-1H-purine-2,6,8(3H)-trione (Fig. S1, ESI†). Gout causes recurring sudden painful attacks for which there is currently no cure without any harmful side effects. In 2012, it was estimated that 1 in 40 people in the UK suffer from gout, with men more likely to suffer than women and risk increasing with age.² Uric acid circulates in the body as the deprotonated urate ion and combines with sodium and water to form monosodium urate monohydrate (MSU) crystals, which are deposited in the joints as principal component in gout stones.

The morphology of MSU is thought to be a particular problem in the body as the needle-like shape of the crystals allows them to “get stuck” in places such as cartilage, which then causes the subsequent inflammation to occur. Consequently, it would be beneficial to develop a method that could directly dissolve or break down the deposited MSU crystals quickly. This would allow for instant alleviation of the symptoms of the patient.

Herein, we present our recent synthesis and characterisation of MSU crystals and investigation of possible methods for dissolution or breakdown of these crystals in a biocompatible aqueous system with a pH value of 7.4, which is similar to the normal value in vivo. Our main goal is to develop a basis for new treatments of gouty arthritis attacks.

MSU crystals were prepared according to an established method.³ 50 cm³ sodium hydroxide (0.2 M) was heated to 70°C. Uric acid (1.67 g, 9.93 mmol) was added in small portions and the solution was stirred for 4 h. A white opaque powder precipitate was recovered by filtrating, washing with distilled water, and drying at 60°C.

Initial characterisation of the crystals was performed by using powder X-ray diffraction (XRD) on a PANatical Empyrean X-ray diffractometer. The XRD pattern (Fig. 1) is in line with previous literature.⁴ All the diffraction peaks can be indexed to the triclinic unit cell of MSU crystals with $a = 10.888$, $b = 9.534$, $c = 3.567$ Å, $\alpha = 95.06^\circ$, $\beta = 99.47^\circ$, and $\gamma = 97.17^\circ$, space group $P\bar{1}$. The intensity of the (011) peak is much higher than other peaks.

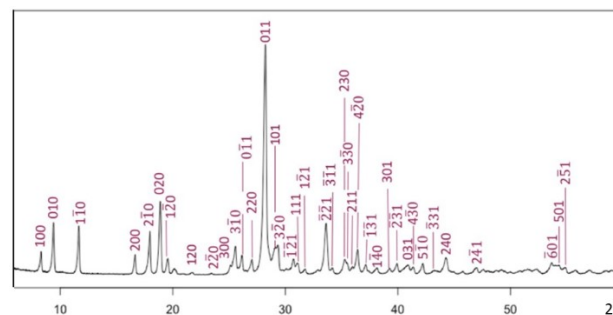


Fig. 1 Powder XRD pattern of the as-synthesised monosodium urate monohydrate crystals.

According to a previous structure refinement by Mandel and Mandel,⁴ the urate anions are hydrogen bonded and aligned along their edges, forming sheets of purine rings parallel to the (011) plane. Sodium ions are located in the inter-sheet space, six coordinating to the urate molecules with a cation- π interaction and water is able to coordinate to the sodium ions and to the negatively charged nitrogen. Fig. 2 shows the structural model revealing these characters when viewing down the [001] and [010] directions. The sheets of purine rings can be better viewed along the [100] direction (Fig. S2, ESI†).

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† Electronic Supplementary Information (ESI) available: Uric acid and MSU molecules, structural model, more TEM and SEM images, EDX spectrum, preparation of buffer solutions. See DOI: 10.1039/x0xx00000x

The crystal size and morphology were examined using scanning electron microscopy (SEM) on a Jeol JSM-5600 or a Jeol JSM-6700F field-emission-gun scanning microscope. The specimens were pre-coated with a thin gold film to reduce electron beam charging effect. A typical SEM image of the MSU product is shown in Fig. 3a. The particles have a straight needle-like morphology with various diameters and lengths. Some larger crystals reach 1 μm in diameter and over 20 μm in length.

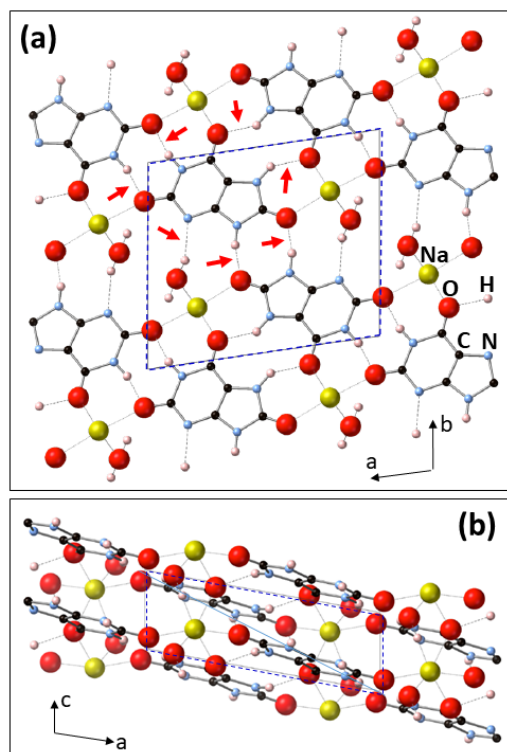


Fig. 2 Structural model of MSU crystal looking down the (a) [001] and (b) [010] zone axes, showing the intermolecular bonding and the stacking of purine layers. The unit cell is outlined by the parallelograms of dash lines. Seven hydrogen bonds around a urate ion are indicated by the red arrows in (a).

To find crystallographic orientation of the crystals, transmission electron microscopy (TEM) was performed on a Jeol JEM-2011 microscope operated at 200 kV. It was found that the hydrated organic crystals were extremely electron beam sensitive and decomposed in seconds under normal conditions for high resolution TEM (HRTEM) (Fig. S3, ESI[†]). In our previous work, we demonstrated that recording HRTEM images of some beam sensitive materials, such as C₆₀/trimethylbenzene composite nanowires⁵ and metal organic framework MOF-5⁶ was possible, if the sample preparation and microscopic conditions were properly controlled.⁷ MSU crystals are even more sensitive to electron beam and no lattice images were recorded. However, the recording of low dose TEM images and selected area electron diffraction (SAED) patterns have been successfully performed.

Fig. 3b shows a TEM image of a MSU crystal with the corresponding SAED pattern (Fig. 3c). Based on the measured d-

spacings of the two marked independent diffraction spots, 1.78 and 4.70 Å with an inter-plane angle of 84.7°, these diffraction spots in the SAED pattern can be indexed into the (002) and (020) planes of the MSU structure. The longitudinal axis of the needle-like particle can be determined to be parallel to the [001] axis of MSU, implying a fast growth along this direction with stacking of the urate anions and sodium cations. On the other hand, the stable facet at the tip of the particles is a sheet of purine rings on the (011) plane, which is not perpendicular to the [001] axis. Therefore the ends of the particles have a inclined plane, as seen in the inset of Fig. 3a and Fig. S4, ESI[†]. This character makes it much easier for the needle crystals to pierce into the soft tissue in the joints. This crystal morphology built up by stacking urate in the (011) planes can explain why the intensity of the (011) peak in XRD (Fig. 1) is so strong. Both the dominant growth along the c axis and the inclined plane at the end can be attributed to the strong inter-sheet connection by the six coordinated Na⁺ cations. If this growth could be inhibited by removing Na⁺, the formation of the needle morphology could be prevented. However, this cannot be achieved in vivo, since significantly reducing the concentration of sodium in human body is not appropriate in practice.

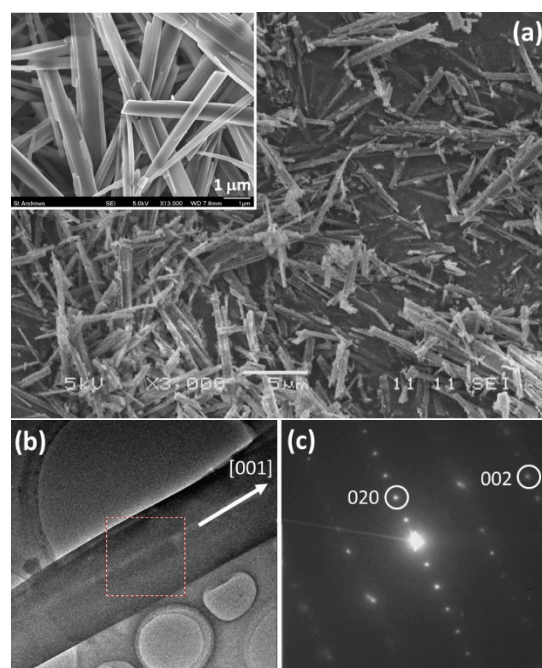


Fig. 3 (a) SEM image of the product of MSU crystals. The inset is an enlarged SEM image. (b) TEM image of a MSU crystal and (c) the corresponding SAED pattern indexed to the MSU crystal structure. The arrow in (b) indicates the longitudinal axis of the needle-like crystal.

If we examine the structure of MSU crystals on the [001] projection (Fig. 2a), we can see indeed that the urate ions are connected to each other side-by-side in the (011) plane by seven hydrogen bonds, a form of dipole-dipole interaction, which may be weakened by the presence of either H⁺ or OH⁻ ions. Instead of breaking up the crystal structure by dissociation of the sodium-urate bonds, it would be beneficial to seek to break the hydrogen bonds in between the urate

ions in the structure. We therefore performed a test of effect of pH value of the solution on dissolution of the MSU crystals.

The first test of dissolution was in NaOH solutions, which were prepared by diluting 1 M NaOH solution with pH of 14 into solutions with pH values from 7.4, and 8 to 13 with intervals of 1. MSU crystals (12 mg, 0.05 mmol) were added to a solution of 5 cm³ for the test.

MSU dissolved in a solution of 1 M sodium hydroxide at room temperature. In the solution with pH of 13, the crystals did not dissolve noticeably at room temperature after 1 h, but were able to dissolve after 4 min at 37°C. In the solution with pH of 12, the crystals dissolved at 37°C after 8 min. The dissolution process is therefore endothermic. The remaining solutions from pH 11 – pH 7.4 were not able to dissolve MSU significantly at room temperature, 37°C or at any temperature up to 50°C within the time limits used.

If allowing all solutions to sit at room temperature overnight, it was noted that the pH 13 solution of NaOH did, in fact, appear to dissolve all crystals. This indicates that the dissolution rate in this solution was very slow. Dissolution rates in other alkaline solutions with pH < 13 were even slower. Nevertheless, the condition of pH value of 13 or higher cannot be applied in vivo.

A series buffer solutions of pH values from 1 to 10 in intervals of 1, and an extra solution at pH = 7.4, were prepared (Table S1, ESI†).⁸ Each solution was diluted to 200 ml with distilled water to give the final buffer. 12 mg crystals were added into a solution of 5 ml for testing its solubility. The study showed that obvious dissolution was only observed in the solutions with pH falling in a range from 1 to 4. The lowest extents of dissolution appeared to be in the range of pH 6 – pH 8. This suggests that MSU is more soluble at high and low pH values and reaches a minimum in solubility around the pH of ~7.4. Consequently, although the formation of MSU crystals is a reversible process, the dissolution of the crystals is very slow in water, which is even slower in a solution containing uric acid and Na⁺.⁹

Another method to break the MSU crystals is using sonication. It has a long history (over 60 years) to use sonication for pain relief in the management of acute attacks. In limited studies, it was reported that sonication could destruct the aggregates of urate crystals¹⁰ and reduce the mean crystal size to a few micrometers.¹¹ However, the mechanism of the reduction of the crystal size was not discussed in depth. It would be more interesting to know whether the MSU crystals could be broken down to nanometer scale or the molecular level. Since the hydrogen bonds in the urate sheets are much weaker than the connection between the urate ions and Na⁺ cations along the [001] zone axis, a sonochemical treatment would break the hydrogen bonds and peel off the crystals along the [001] direction strip-by-strip. The product would be long chain polymerised molecules consisting of urate ions connected by Na⁺ cations.

12 mg MSU crystals were added into the buffer solution with pH 7.4. The crystals slowly sunk to the bottom of the container. The specimen was placed in a sonication bath (T80, Agar Scientific, 40 kHz). The samples were sonicated for intervals of 15 min for up to 2 h.

For a XRD study, the sample was transferred into a capillary (0.5 mm in diameter). XRD pattern was collected on a STOE STADIP diffractometer using Cu K_{α1} X-ray radiation. The beam size was 1 mm, much larger than the capillary. Data collection was made in a scanning range of 2θ from 10° to 80° in 2 h with steps of 0.01°. Fig. 4a shows XRD patterns from the MSU samples after sonication for

different times. After sonication treatment for 15 min, the majority of MSU crystals turned to be a non-crystalline phase with small amount of remaining MSU crystals, showing reduced intensities of the diffraction peaks as seen in pattern (II) in Fig. 4a. Increasing the sonication time to 45 min and 105 min, the intensities of the diffraction peaks of MSU further reduced (Fig. 4a III and IV).

We noted that the intensity of the X-ray scattering from non-crystalline component also reduced with the longer sonication. Even we tried to fill the powder in the capillary as much as possible, the density of the powder might decrease with sonication because the ordering of the polymerised molecules reduced. We therefore calculated the intensity (peak area) ratios of the (011) peak to the polymer component (large bump from 15° to 40°), (II) 0.106, (III) 0.080 and (IV) 0.063. Considering that part of the polymerised molecules were further decomposed into small molecules as discussed below, we can conclude with more confidence that the proportion of the MSU crystals in the powder samples reduces under the sonication.

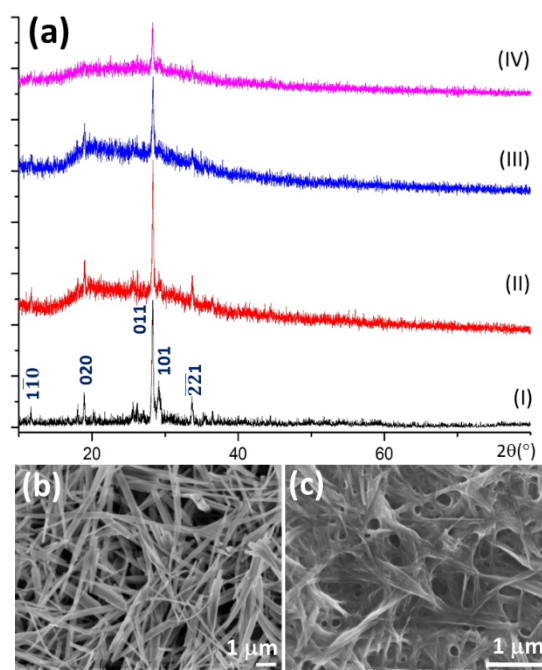


Fig. 4 (a) XRD patterns of MSU precipitates using the same diffraction conditions, (I) before sonication, and after sonication in the buffer solution with pH of 7.4 for (II) 15, (III) 45, (IV) 105 min. SEM images of MSU crystals isolated from the non-crystalline component after sonication for (b) 45 min and (c) 2 h.

Fig. 4b shows a SEM image of a cluster of MSU crystals isolated from the non-crystalline component after sonication for 45 min. The diameter of the needle-like crystals is reduced with some very fine and soft nanowires also visible, providing clear evidence of the peeling mechanism of the MSU crystals along the longitudinal axis by sonication. Consequently, the reduction of the diffraction peaks of MSU crystals in this sample can be attributed to both a reduction of the crystal size and a morphology change from straight needles to soft nanowires.

A longer sonication treatment cleaved the MSU further. As seen in Fig. 4c, after sonication for 2 h, a cluster of the crystals shows even thinner nanowires, which are completely covered by a thick layer of disordered soft matter. As confirmed below, these thin crystalline

nanowires are protected by the polymerised MSU molecules and their further destruction becomes difficult, unless the polymer coating is dissolved. Nevertheless, a change of most MSU crystals into polymerised molecules can certainly increase the dissolution rate of the crystals.

The appearance of polymerised MSU molecules in an aqueous solution is different from that of original needle crystals. As shown in Fig. S5a, ESI⁺, the non-sonicated MSU needle crystals sink to the bottom of the solution, while a thick gel-like layer of precipitate forms after sonication for 15 min. In other words, the volume of the precipitate increases significantly after a sonication treatment.

The major component of the sonicated sample is non-crystalline soft matter. Fig. S5b (ESI⁺) shows an SEM image of a sample after sonication for 15 min. A few MSU crystals are embedded in a piece of thick gel-like precipitate. Fig. S5c (ESI⁺) is another SEM image from a thin piece of non-crystalline component of the sample after sonication for 45 min. Again, some thin straight needle-like crystals in bright contrast can be identified.

An even thinner layer of the non-crystalline component without any crystalline particles was selected for further investigation. Fig. S6 (ESI⁺) shows a TEM image of a thin polymer-like film from a sonicated MSU sample. The even image contrast indicates no embedded MSU crystals. The corresponding SAED pattern (Top right corner of Fig. S6, ESI⁺) shows no any diffraction spots. On the other hand, energy dispersive X-ray (EDX) microanalysis reveals significant amounts of elements of C, N, O, and Na, indicating that this non-crystalline component is actually polymerised MSU molecules.

When the polymerised MSU molecules become very small, they are dissolved into the solution. Mass spectroscopy of the clear solution in a sonicated sample shows urate molecules in different forms. For example peaks of the Mass spectra indicate monomer $C_5H_4N_3O_3^-$ (UA⁻), $M/Z = 167.0207$ (calculated value, cal. = 167.0205), dimer $UA^-H^+-UA^-$, $M/Z = 335.0499$ (cal. = 335.0488), dimer $UA^-Na^+-UA^-$, $M/Z = 357.0318$ (cal. = 357.0308), trimer $UA^-H^+-Na^+-UA^-Na^+-UA^-H^+$, $M/Z = 549.0564$ (cal. = 549.0567), etc.

In conclusion, as we demonstrated in the present work, because the urate anions in MSU crystals are weakly connected by hydrogen bonding in the (011) planes and strongly connected by Na⁺ cations along the c-axis, the crystals can be peeled off during a sonochemical treatment into some long chain polymerised MSU molecules. This fundamental research sheds light on future development of a physical therapy for breaking down gout stones into non-crystalline polymerised molecules and finally dissolving them completely.

Conflicts of interest

There are no conflicts to declare.

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Needle-shaped crystals of monosodium urate monohydrate, the principal component in gout stones, were peeled off into polymerised molecules by sonication.

